

Restriction Enzyme Digest and Dephosphorylation

For 50 μ L reactions:

1. Assemble reaction in a thin walled tube in the following order:

Plasmid DNA(~1ug)	x μ L
NEB CutSmart Buffer	5 μ L
EcoR1	1 μ L
Pst1	1 u
ddH2O	x μ L

2. Incubate reaction at 37°C for 1 h.
3. Heat kill enzyme at 80°C for 10 min.
4. Add 1ul of Shrimp Alkaline Phosphatase to plasmid backbone (pSB1C3)
5. Incubate reaction at 37°C for 1 h.
6. Heat kill enzyme at 80°C for 10 min.
7. Follow BioBasic enzymatic clean-up protocol